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THE EFFECTS OF ATMOSPHERIC PRESSURE AND COMPOSITION ON THE FLIGHT OF DROSOPHILA

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In the absence of a satisfactory theory of minute airfoils, not to mention airfoils which like the insect wing undergo continuous and variable angular motion, only limited help in the detailed analysis of insect flight can be obtained at present from the science of aerodynamics. Nevertheless, it is worthwhile to attempt to apply certain elementary aerodynamic concepts in an effort to develop a rational basis for the study of the flight process.

One such approach is to regard the wings of an insect as diminutive paddles which in each wingbeat serve to impart a specific average velocity to a specific mass of air. This point of view has already proved useful in analyzing the correlation between frequency of wingbeat and the dimensions of the wings and thoracic muscles in various species of *Drosophila* (Reed, Williams and Chadwick, 1942). Considering flight of an insect in these simple terms, the power output (P) should be proportional to the mass of air moved per beat (m), the square of the average velocity (v) imparted to this mass, and the wingbeat frequency (f):

$$P \propto mv^2f. \quad (1)$$

For present purposes the relationship may be further simplified, since

$$v \propto f$$

and, therefore, substituting in Equation (1),

$$P \propto mf^3.$$

or

$$f^3 \propto P/m. \quad (2)$$

Thus, according to this analysis, the cube of wingbeat frequency should be proportional to the power output divided by the mass of air moved per beat. That this relationship has real validity is indicated by correlations already demonstrated between wingbeat frequency and the energy consumption during flight. In the case of *Drosophila* the flight energy (oxygen consumption and carbon dioxide production) was found actually to vary as the cube of wingbeat frequency (Chadwick and Gilmour, 1940; Chadwick, 1947). Manifestly, such a correlation would be expected on the basis of Equation (2), provided that the mass of air moved in each remained constant.

While the basic relationship has then been verified experimentally, the effects of varying the mass of air moved per beat have in contrast remained very largely a matter of speculation. V. Buddenbrock (1919), Roch (1922) and Sotavalta (1947) adopted the simplest means of altering this factor by clipping the tips from the wings. Though these investigators have offered various interpretations of their results, the increase in wingbeat frequency they observed seems to us attributable directly to the decreased mass of air moved per beat. However, the quantitative aspects are not readily established in experiments of this sort, which we have also performed, for the reasons that surfaces of equal area from different parts of the wing may not be equivalent aerodynamically and that we have as yet no means of measuring their presumably different contributions to the outflowing air stream. Thus, while one may observe that progressively shortening the wings results in a progressive increase in the rate of wingbeat, one is unable to derive a precise statement of the relationship thereby revealed. Such experiments suffer also, of course, from the necessity of mutilating the structure one is attempting to study.

Fortunately these difficulties can be avoided relatively easily, since, if the wings are regarded as sweeping out a specific volume of air with each beat, the mass of air moved is obviously dependent on the gaseous density of the medium. By varying the density, the wings may be made to sweep out a volume whose mass may be altered continuously, and the resulting changes in performance correlated, in terms of wingbeat frequency, with the density change. Alterations of density are produced and measured conveniently merely by varying the pressure of the air in which the insect flies. Our problem resolves itself then into an examination of the effects of changes in atmospheric pressure on the rate of wingbeat.

As far as we are aware, there has been no adequate investigation of this matter. Magnan (1934) states that "frequency changes with pressure also. Thus a fly making 160 strokes per second makes 20 more when placed in a vacuum corresponding to an altitude of 2000 meters." By an acoustic method Sotavalta (1947) determined the wingbeat frequencies of several species of bees at a series of subatmospheric pressures, but observed no deviations from the rates measured under normal conditions. Case and Haldane (1941) note in passing that *Drosophila*, exposed to an air pressure of 10 atmospheres, was unable to fly. These limited observations are all we have been able to find in the literature.

Our initial and simple objective of studying the effects of variation in atmospheric pressure has, as is so frequently the case, raised more problems than were contemplated at the outset. For, in addition to altering density, variations in air pressure produce systematic changes in oxygen tension and in pressure as such. On this account, efforts to study each of these factors separately were necessary. Measurements of the oxygen consumption during flight at normal and reduced pressures were also made when it became apparent that, for an understanding of the other data, more information was needed about the energy relationships concerned.

MATERIALS AND METHODS

A. Measurements of wingbeat frequency

Our first experiments were performed on *Drosophila repleta* Wollaston, a species particularly adapted to these studies on account of its dependable tarsal flight

reflex. Later, *D. virilis* Sturtevant was also used. In working with *D. repleta*, individuals were selected at random from a wild population that maintained itself in the animal rooms and were used without regard to age or sex, since we were unaware at the time that frequency of wingbeat is determined to some extent by these variables. This defect in technique has contributed to the scatter in the data, and was avoided in the studies with *D. virilis*, which were grown under standard conditions at 25 degrees C. and isolated daily on emergence.

The apparatus in which wingbeat frequency was measured is a simplified version of the flight chamber described by Williams and Chadwick (1943), and has been diagrammed in Figure 1. It consists of a glass pressure chamber whose temperature was controlled either by circulation of water through a surrounding jacket or by immersion in a constant temperature bath. Provisions were made for clamping

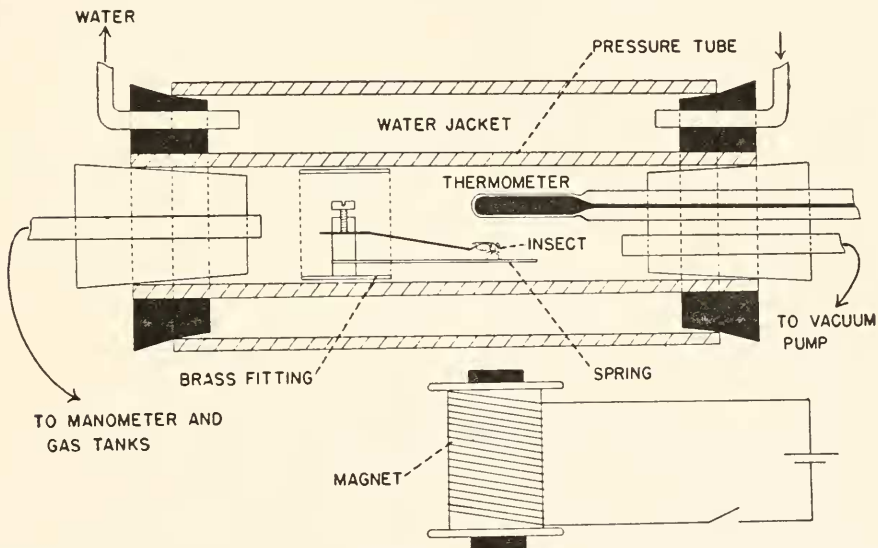


FIGURE 1. Apparatus for measurement of wingbeat frequency at various pressures. For explanation see text.

rubber stoppers, one of which held a thermometer, into the ends of the pressure chamber, while glass tubes, passing through the stoppers, allowed gas mixtures of known composition to be circulated.

These mixtures were made up at high pressure in commercial gas cylinders and analyzed before use. At each change of gas mixture the chamber was washed thoroughly with the succeeding mixture. Pressure within the chamber was varied by the addition of compressed gases or by means of a vacuum pump. A pressure gauge and mercury manometer, measuring up to 5 atmospheres, were sealed into the gas lines and permitted a continuous check on the pressure within the experimental chamber. Relative humidity was held at or near 100 per cent by placing a few drops of water within the chamber and by bubbling the gases through water as they entered.

The rate at which pressures were altered between successive sets of measurements had no obvious effect on wingbeat frequency. Ordinarily these changes were made fairly quickly and though several minutes were then allowed for equilibration even this period of adaptation appeared unnecessary. It was possible therefore to test the various pressures in rapid random succession.

All the data obtained with *D. repleta* have been computed in terms of the response at 25 degrees C. In some of the earlier experiments the measurements were made at temperatures that deviated slightly from 25 degrees C.; these have been adjusted by applying a factor derived from studies of the effect of temperature on wingbeat frequency. Although the validity of this treatment was tested and confirmed, it became unnecessary in all later experiments when the flight chamber was maintained at 25 ± 0.1 degrees C. With *D. virilis*, various constant temperatures were used, as stated in the tabulation of results.

Measurements of wingbeat frequency were made on fastened specimens according to the method previously described. To evoke and terminate the flight of the insect within the sealed chamber, the tarsal reflex was utilized. Flight was induced by withdrawing a spring platform from under the animal's feet and stopped by interrupting the current to the electromagnet shown in Figure 1.

Frequency of wingbeat was measured by means of a General Radio "Strobotac." In each instance, the maximal frequency, occurring within the first few seconds of flight, was recorded. Each flight was therefore extremely brief, with a duration in most cases of about 2 seconds. In this way the onset of fatigue was postponed, so that several hundred measurements could be made on most individuals. Determinations were made at intervals of 10 seconds, the 8 seconds of rest between flights having been found adequate for recovery. The response of each individual to each experimental condition was usually recorded as the mean of 20 measurements.

Each fly was tested initially in air at atmospheric pressure and then under a variety of experimental conditions. At intervals during an experiment the performance was rechecked in air; any animal showing significant deviation from its initial response was discarded. Since it was impossible to test all individuals under all circumstances and since there were considerable differences in the wingbeat frequencies of different individuals in air at 760 mm. Hg (mainly because of our ignorance of the influence of age and sex), results for *D. repleta* have been calculated in terms of the deviation in frequency under each set of conditions from the frequency observed for that individual in air at 760 mm. Hg. For presentation of the average data as in Tables 1, 3, and 6, the average deviations in wingbeat frequency at each pressure have been added to or subtracted from the mean frequency for all individuals at 760 mm. Hg in air, in order to provide a more direct comparison of the rates in the various media used. Thus, for example, the average frequency of 10,700 cycles per minute at 3860 mm. Hg shown in Table 1 was obtained by subtracting 1990, the average decrement for these 17 animals from their rates at 760 mm., from 12,690, the mean for all 72 flies at normal pressure. The statistically preferable procedure of using a different randomly selected sample of flies for each set of conditions would have been impractical, particularly since we had not then succeeded in establishing *D. repleta* in culture.

With *D. virilis*, a series of only 4 or 5 pressures was used, and each insect was flown 10 times at each pressure. The results thus obtained were averaged and are presented in this form in Table 2.

B. Measurements of oxygen consumption during flight

Details of the technique for measuring oxygen consumption of *Drosophila* in flight have been described in previous reports (Chadwick and Gilmour, 1940; Chadwick, 1947). In the present study, differential volumeters (Fenn) were used and an arrangement adopted which allowed simultaneous evacuation of both vessels, as shown in Figure 2. The vessels had capacities of about 13 ml. and were connected by a capillary with a volume of about 5 cu.mm. per cm.

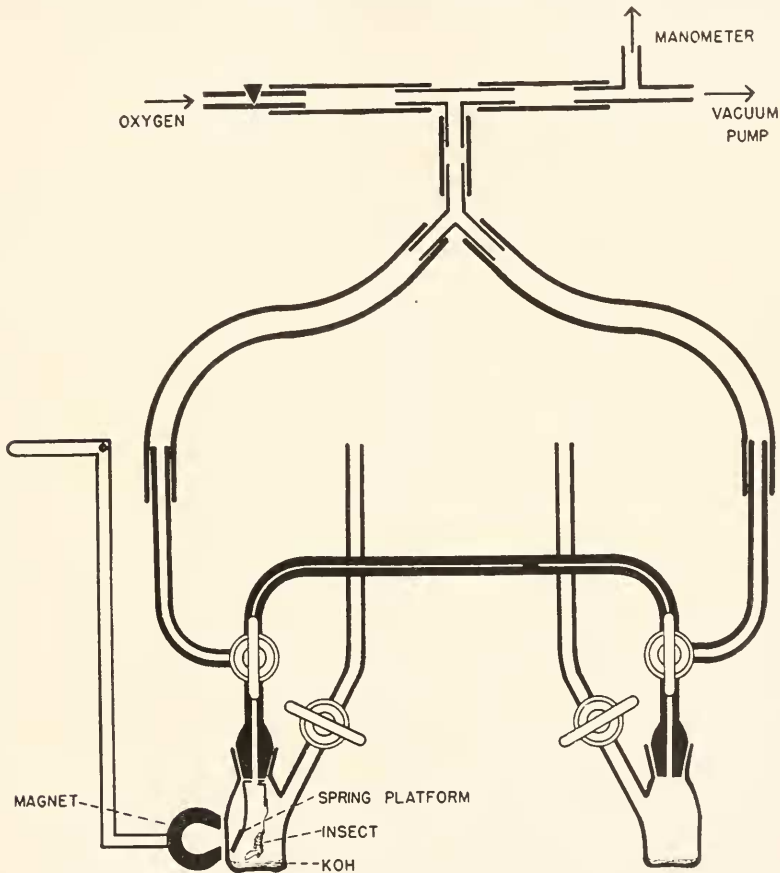


FIGURE 2. Apparatus for measurement of oxygen consumption during flight at normal and reduced pressures. For explanation see text.

Individual *D. virilis* of known age were anesthetized with carbon dioxide gas and fastened with paraffin to a fine wire which was then attached to the head of the respirometer. The fly was suspended head-down in the vessel, its feet in contact with the usual retractible platform. To depress the paraffin-coated platform and induce flight, a small permanent magnet was brought up to the side of the vessel in the bath. Carbon dioxide given off by the insect was absorbed in 0.1 ml.

of 15 per cent KOH in the bottom of the vessel, which had been fitted with a sleeve of filter paper to increase the absorbing surface.

During a 20-minute period of equilibration at 19.3 ± 0.01 degrees C. in the water bath, both vessels were gassed out with oxygen from a commercial cylinder. The stopcocks were then turned to running position and the resting oxygen consumption measured for 30 minutes or longer. In about half the experiments, flight was then induced at normal pressure and allowed to continue for 10 or 20 minutes, during which time readings of oxygen consumption were taken every minute and measurements of the rate of wingbeat every 10 seconds. For the latter, the specimen was viewed in silhouette against the flash lamp, which had been let down into the bath

TABLE 1
Wingbeat frequency as a function of air pressure and density
Drosophila repleta in moist air at 25° C.

Air pressure mm. Hg	Density gms./liter	Average wingbeat frequency beats/min.	Number of specimens	Number of measurements
3860	6.00	10,700	17	322
3450	5.36	10,910	22	442
3100	4.82	10,990	25	525
2820	4.38	10,940	21	430
2580	4.01	11,330	20	425
2320	3.60	11,330	20	410
2100	3.26	11,660	21	425
1880	2.93	11,570	15	270
1660	2.57	11,720	13	224
1380	2.14	12,050	13	216
1200	1.86	12,110	14	250
980	1.51	12,320	15	310
760	1.17	12,690	72	2730
680	1.05	12,880	22	399
600	0.92	12,930	20	358
500	0.77	13,070	20	390
400	0.61	13,210	37	770
300	0.45	13,310	20	353
260	0.39	13,540	19	824
200	0.30	13,680	32	615
140	0.20	13,950	18	347
100	0.14	14,060	14	237

inside a glass cylinder. After the measurements at normal pressure, the system was evacuated to 200 or 400 mm. Hg, and the same procedure repeated. In the remaining experiments, the order of pressures was reversed; for example, the first set of measurements was made at 200 or 400 mm. Hg, and the second at normal pressure.

Careful attention to the lubrication and seating of stopcocks and other joints was essential since an inward leak amounting to a fraction of a cu.mm. per minute could render the measurements of oxygen consumption at low pressures valueless. Vaseline was used successfully as a stopcock grease at temperatures of 20 degrees C. or less, but although this and several other lubricants were tried, attempts to repeat

these experiments at 26 degrees C. failed because leaks around the stopcocks invariably developed before a run could be completed. Only those experiments were considered valid in which a reasonably constant rate of resting oxygen consumption, of a reasonable magnitude in comparison with earlier measurements, was obtained for at least one half-hour before flight at each pressure, and in which the rate of oxygen consumption returned to and maintained a value approximating the preflight level within a few minutes after flight had ceased.

Although it was impossible in the system diagrammed to be certain that leakage was zero, one can state with assurance that any leaks which did occur were not greater than the average resting rate of about 30 cu.mm. per gm. per minute. Since the flight oxygen consumption was computed by subtracting the resting rate from the total measured during the flight which followed immediately, such errors would

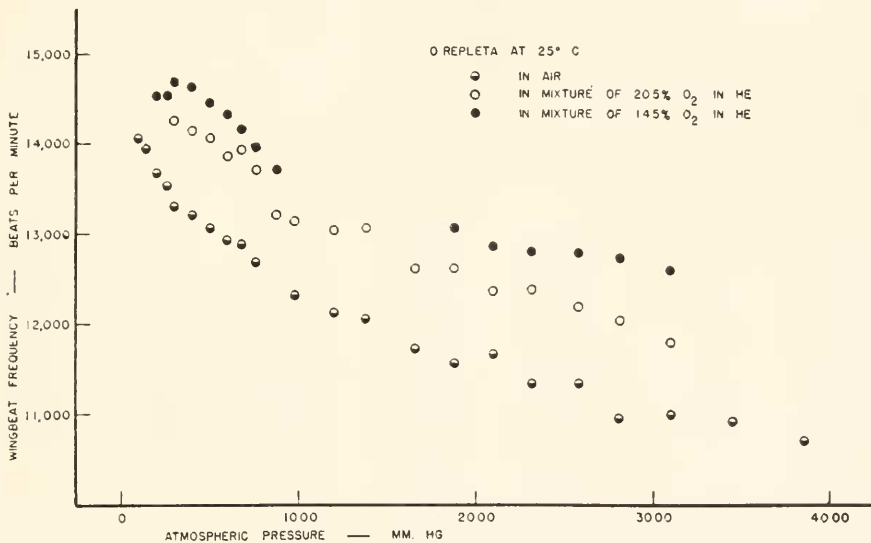


FIGURE 3. Wingbeat frequency of *D. repleta* as a function of atmospheric pressure in air and in two oxygen-helium mixtures.

affect mainly the resting rates rather than the flight respiration in which we were chiefly interested. However, if one makes the highly conservative allowance of a possible error of 30 cu.mm. per gm. per minute, this will amount only to some 8-9 per cent of the average flight oxygen consumption measured at 19.3 degrees C. and will not alter significantly the conclusions we have drawn from the data.

Difficulty was experienced in some of the early attempts in obtaining a flight response from the insect after the vessels had been lowered into the bath. Illuminating the experimental vessel with a 40-watt bulb in a reflector at the side of the bath overcame this trouble. The lamp neither interfered with the observations of wing movement nor, since the glass wall of the bath and at least 6 inches of well-stirred water separated it from the respirometer, disturbed the measurements of oxygen consumption. In blank runs, no movement of the index drop occurred as a result of turning the lamp or stroboscope on or off.

RESULTS

A. Observations of wingbeat frequency

In order to have a means of differentiating between effects due to variation in total pressure, oxygen tension and gaseous density, *D. repleta* was tested in 5 different media: (1) air, (2) 14.7 per cent oxygen in nitrogen, (3) nitrogen-oxygen mixtures of higher oxygen content than air, (4) 14.5 per cent oxygen in helium and (5) 20.5 per cent oxygen in helium. The rate of wingbeat of *D. virilis* was measured in air at 5 different pressures, both at 19.3 degrees C. and at 25.9 degrees C.; and at normal pressure, 25.9 degrees C., in a mixture of 6.1 per cent oxygen in nitrogen.

TABLE 2
Wingbeat frequency of D. virilis as a function of air pressure

Pressure in mm. Hg.		1520	760	400	200	100
Specimen number	Age in days	Wingbeat frequency in beats per minute				
a. Moist air at 19.3 degrees C.						
3-112 ♀	8-9	10,690	11,000	11,680	12,660	13,180
3-113 ♀	8-9	8,740	9,550	10,390	11,280	refused
3-151 ♀	12-13	11,120	11,470	12,060	12,980	refused
3-152 ♀	12-13	10,410	11,100	11,930	12,770	refused
3-153 ♀	12-13	10,100	11,150	11,650	12,220	refused
3-155 ♀	12-13	10,570	11,290	12,300	13,390	14,020
3-157 ♀	12-13	10,630	10,870	11,620	12,560	refused
3-158 ♀	12-13	9,210	10,380	10,630	11,500	refused
3-159 ♀	12-13	10,950	11,120	11,720	12,030	13,060
3-1512 ♀	12-13	11,030	11,390	11,960	12,750	13,380
Average (10 flies)		10,350	10,930	11,590	12,410	—
Average (4 flies which flew at 100 mm.)		10,810	11,200	11,920	12,710	13,410
b. Moist air at 25.9 degrees C.						
3-211 ♀	4-5	13,710	14,010	14,870	15,670	16,370
3-212 ♀	4-5	13,870	14,290	14,770	15,590	16,130
3-213 ♀	4-5	14,250	14,510	14,810	15,670	15,790
3-214 ♀	4-5	13,650	13,990	14,290	14,650	15,150
3-215 ♀	4-5	13,450	14,050	14,710	15,550	15,970
3-217 ♀	4-5	14,170	14,590	15,290	16,010	16,250
3-218 ♀	4-5	13,790	14,210	14,930	15,670	15,850
3-221 ♀	5-6	12,050	12,880	14,090	15,510	refused
3-222 ♀	5-6	12,970	13,590	14,530	15,630	16,050
3-223 ♀	5-6	10,320	10,950	12,330	13,370	13,940
Average (10 flies)		13,250	13,710	14,460	15,330	—
Average (9 flies which flew at 100 mm.)		13,350	13,800	14,500	15,310	15,720

Each observation is the mean of 10 measurements.

1. Air

The effects of variation in air pressure on the frequency of wingbeat of *D. repleta* were studied in a total of 72 individuals, over a range of 5 atmospheres. As indicated in Table 1 and Figure 3, the frequency of wingbeat decreased gradually as the pressure increased. This effect was observed over the whole range of pressures investigated, from 80–100 mm. Hg, below which the animals failed to fly when stimulated, to a pressure of 3860 mm. Hg.

Examination of the data reveals an apparent discontinuity in the relationship at about 680 mm. Hg. This is best visualized on the logarithmic grid of Figure 4. Our reasons for considering it an artefact are given in the discussion. No such discontinuity is evident in the data obtained at two temperatures with *D. virilis* (Table 2 and Figure 4).

TABLE 3

Wingbeat frequency as a function of pressure in a mixture of 14.7 per cent oxygen in nitrogen Drosophila repleta at 25° C.

Total pressure mm. Hg	Density gms./liter	Average wingbeat frequency beats/min.	Number of specimens	Number of measurements
3100	4.78	10,950	12	202
2820	4.34	11,090	7	140
2320	3.57	11,480	8	87
1880	2.89	11,610	12	190
1380	2.12	11,960	18	290
980	1.50	12,220	19	370
760	1.16	12,600	39	1197
680	1.04	12,860	12	170
600	0.91	12,890	5	70
500	0.76	13,300	11	157
400	0.60	13,260	9	140
300	0.45	13,540	10	146
200	0.30	13,970	4	55

2. 14.7 per cent oxygen in nitrogen

A further series of 39 *D. repleta* was tested in a mixture of subnormal oxygen content in order to emphasize the possible effects of decreased oxygen tension at low total pressures. As shown in Table 3 and Figure 4, no significant difference was evident in comparison with the relationship observed in air. Comparative data for *D. virilis* in air and in 6.1 per cent oxygen in nitrogen are given in Table 4. Again no difference was observed.

3. Atmospheres of high oxygen content

By somewhat different measures the effects of subatmospheric pressures of mixtures with high oxygen content were studied. In these experiments each individual was tested at a specific low pressure in air and then subjected to the same pressure in an atmosphere rich in oxygen. The two sets of measurements, samples of which are given in Table 5, showed no significant differences. It was never possible to

cause the frequency of wingbeat to rise above the value in air by supplying a greater than normal proportion of oxygen.

4. Helium-oxygen mixtures

Having found no correlation between the tension of oxygen and the response of the insect to pressure, there remained the problem of distinguishing between the two other variables involved in these experiments; namely, gaseous density and pressure *per se*. Their separation seemed difficult at first, since the density and total pressure of a given gas mixture are directly proportional. However, the fact that helium is an inert gas with a density only about one-seventh that of nitrogen offered a means of attacking the problem. Using helium and oxygen, mixtures

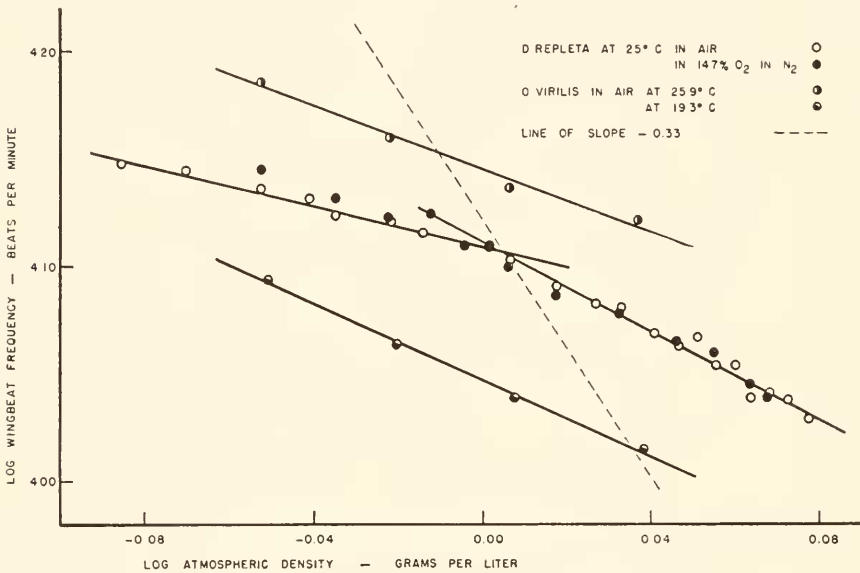


FIGURE 4. Wingbeat frequency of *D. repleta* and *D. virilis* as a function of atmospheric density. Solid lines fitted to empirical data by the method of least squares. Broken line of slope -0.33 added for comparison.

may be prepared which differ from air or other oxygen-nitrogen mixtures in density but not, presumably, in regard to most other properties which are physiologically significant.

Two helium-oxygen mixtures were used for this purpose, one containing 14.5 per cent oxygen, the other 20.5 per cent. At 25 degrees C. and one atmosphere, the densities of these mixtures, both of which contained water vapor and small amounts of nitrogen, were approximately 0.33 and 0.39 grams per liter, respectively, as compared with 1.17 grams per liter, the density of moist air.

Frequency of wingbeat was measured with *D. repleta* in each of these mixtures throughout most of the range of pressures with results recorded in Table 6 and Figure 3. It is evident that the frequency was higher in either mixture than in

TABLE 4

Wingbeat frequency of D. virilis as a function of oxygen tension
Moist gas at 25.9 degrees C. and 760 mm. Hg

Specimen number	Age days	Wingbeat frequency in	
		Air	6.1 per cent O ₂ in N ₂
		beats per minute	
3-214 ♀	4-5	13,240	13,220
3-215 ♀	4-5	13,300	13,180
3-217 ♀	4-5	13,840	13,940
3-218 ♀	4-5	13,460	13,120
3-221 ♀	5-6	12,900	12,900
3-222 ♀	5-6	13,700	13,820
3-223 ♀	5-6	10,740	10,690*
3-232 ♀	6-7	12,670	12,710
3-234 ♀	6-7	11,730	11,950
3-235 ♀	6-7	13,140	13,210
Average (10 flies)		12,870	12,870

* Rate of this specimen apparently depressed by previous flights; initially the rate was 10,950 in air at 760 mm. Hg (Table 2).

Each observation is the mean of 10 measurements.

air of the same pressure, and that it was highest at any given total pressure in the mixture having the least density.

During these experiments the animals reacted badly to the helium mixtures. It was frequently difficult or impossible to induce flight with the usual stimulus, and, after a relatively small number of flights, the wingbeat frequency began to decrease. The behavior resembled that of a fatigued animal in air, and on this account it was

TABLE 5

Wingbeat frequency in air and in atmospheres with greater oxygen content
Drosophila repleta at 25° C.

Specimen number	Pressure mm. Hg	Wingbeat frequency in		Oxygen content of mixture per cent
		Air beats/min.	O ₂ -N ₂ Mixture beats/min.	
97	1660	13,060	12,590	100
87	680	14,310	14,000	100
93	600	12,060*	11,980	100
90	400	13,430	13,390	44
97	200	14,440	14,440	100
67	200	15,080	14,370	50
64	100	16,380	15,870	50

* In 3.9 per cent oxygen in nitrogen.

Each frequency datum is the mean of 20 observations. Atmospheres were saturated with water vapor at 25° C.

necessary to reduce the number of flights. Thus the points determined for individual specimens during this series of experiments have been averaged as a rule from only five measurements at each pressure. Specimens which had become re-

TABLE 6
Wingbeat frequency as a function of pressure and density in helium-oxygen mixtures
Drosophila repleta at 25° C.

Total pressure mm. Hg	Density gms./liter	Average wingbeat frequency beats/min.	Number of specimens	Number of measurements
a. 14.5 per cent oxygen in helium				
3100	1.34	12,590	10	50
2820	1.22	12,730	9	45
2580	1.12	12,780	9	45
2320	1.00	12,800	8	40
2100	0.91	12,860	8	40
1880	0.81	13,070	8	40
880	0.38	13,720	9	45
760	0.33	13,960	15	75
680	0.29	14,160	5	25
600	0.26	14,330	5	25
500	0.21	14,460	7	35
400	0.17	14,640	7	35
300	0.13	14,680	6	30
260	0.11	14,530	5	25
200	0.08	14,530	5	25
b. 20.5 per cent oxygen in helium				
3100	1.62	11,780	7	50
2820	1.47	12,030	7	50
2580	1.35	12,190	8	55
2320	1.21	12,390	7	40
2100	1.10	12,370	7	35
1880	0.98	12,610	8	40
1660	0.87	12,610	7	45
1380	0.72	13,070	8	35
1200	0.62	13,040	7	35
980	0.51	13,140	9	45
880	0.46	13,210	8	40
760	0.39	13,710	22	135
680	0.35	13,930	8	40
600	0.31	13,870	10	50
500	0.26	14,060	11	55
400	0.21	14,150	9	45
300	0.15	14,260	7	35

fractory in the helium mixtures resumed a normal behavior when returned to air. It was observed also that the animals responded more readily in the helium mixtures when the total pressure was high than when it was one atmosphere or less.

5. Other observations

a. Humidity. In an early series of experiments we found that the frequency of wingbeat failed to increase at subatmospheric pressures when the relative humidity within the flight chamber was low. On the contrary, the rate decreased rapidly and the specimens soon became incapacitated. This effect seems explicable in terms of damage to the insect from loss of water; possibly this factor may account for the negative results reported by Sotavalta (1947).

b. Stroke amplitude. During measurements at high pressures a reduction in the stroke amplitude was evident in most individuals. Though the magnitude of

TABLE 7
Wingbeat frequency of D. repleta before and after removal of halteres

Specimen number	Wingbeat frequency in beats per minute after treatment indicated		
	Etherized and mounted	Re-etherized	Again re-etherized and halteres removed
48 ♀	10,220	10,190	10,250
49 ♂	9,640	9,800	9,930
50 ♂	9,950	10,130	9,890
54 ♀	10,660	10,670	10,490
55 ♀	10,340	10,340	10,380
56 ♀	12,290	12,240	12,280
57 ♀	12,360	12,350	12,120
58 ♀	10,880	10,930	10,610
59 ♀	11,510	11,600	11,720
60 ♀	11,850	11,940	11,880
61 ♀	10,520	10,540	10,380
62 ♀	11,110	11,090	11,580
64 ♂	10,160	10,260	10,320
65 ♀	11,250	11,220	11,450
66 ♀	10,360	10,530	10,520
67 ♀	10,320	10,340	10,640
68 ♀	11,150	11,120	11,210
Average	10,850	10,900	10,920
Standard error	±194	±180	±188

Each datum is the average of 20 determinations. The experiments were run in moist air at 20° C. and 615 mm. Hg.

these changes was not measured, they are of considerable importance theoretically, as will be brought out in the discussion.

c. Halteres. An important rôle of the halteres in regulating the wingstroke has been proposed frequently in the past and reemphasized recently by Pringle (1948), so that it seemed advisable to give some attention to these organs under the conditions of our experiments. As originally reported by Williams and Reed (1944) and subsequently confirmed by Pringle (1948), the halteres are vibrated during flight at the same frequency as the wings, but in opposite phase. See also Curran (1948). This synchrony persists at all pressures. Furthermore, wingbeat frequency in air at various pressures was unaffected by amputation of the halteres as

illustrated by the example given in Table 7. Even when the rate had been increased by clipping the wings, amputation of the halteres had no further effect (Table 8). The relationship between pressure and wingbeat frequency was apparently identical in the case of normal and haltereless flies; in fact, the only difference we have seen in the wing action of the two groups is in the somewhat steadier wingbeat frequency of haltereless flies during continuous flight.

d. Failure to respond at low pressures. In experiments at reduced pressure it was never possible to evoke flight when the total pressure was less than 80 to 100 mm. Hg. Of this pressure approximately 24 mm. Hg must be assigned to water vapor so that, in air, the partial pressure of oxygen amounted only to some 12 to 16 mm. Hg. Under such circumstances one might suppose that failure to fly was due to oxygen lack. Yet flight at lower pressures was still unobtainable when pure

TABLE 8

Wingbeat frequency of D. repleta before and after clipping wings and removing halteres

Specimen number	Wingbeat frequency in beats per minute after treatment indicated				
	Etherized and mounted	One wing clipped	Both wings clipped	Re-etherized	Again re-etherized and halteres removed
34 ♂	12,230	12,980	14,170	14,050	14,010
37 ♀	12,680	13,520	14,930	15,440	15,150
38 ♀	11,650	12,200	12,560	12,680	12,910
39 ♀	11,880	12,880	13,360	13,260	13,460
40 ♀	11,460	12,590	12,810	12,900	13,310
41 ♂	11,340	12,120	12,730	12,710	12,810
43 ♂	11,600	11,800	12,330	12,400	12,150
Average	11,850	12,580	13,270	13,750	13,400

Each datum is the average of 10 determinations. The experiments were run in moist air at 20° C. and 615 mm. Hg, except for those with Specimen Number 34, which were run at 22° C. and 645 mm. Hg. The observations are in contradiction with the finding of Roch (1922) that clipping one wing leaves the wingbeat frequency unaltered. Particular care was taken to cut the same amount from each wing, as nearly as possible; in general, from one-quarter to one-half the wing was removed, by a transverse cut.

oxygen was substituted for air. This rather surprising observation was verified repeatedly.

e. Limiting tension of oxygen. Although some factor other than oxygen lack appears to prevent a flight response at total pressures below about 80 to 100 mm. Hg, there is also a lower limit to the oxygen tension consistent with brief interrupted flight. This is usually encountered when the oxygen tension in the gas mixtures is reduced below 15 to 20 mm. Hg. Thus in the case of air the limitation due to lowering the partial pressure of oxygen is about the same as that imposed by the unknown factor noted in the previous paragraph.

B. Measurements of oxygen consumption

Respiratory rates averaged over 10 or 20 minutes of continuous flight at normal and reduced pressure, together with the average rates of wingbeat observed simul-

TABLE 9

Oxygen consumption and wingbeat frequency of D. virilis during continuous flight at normal and reduced pressures in oxygen

Specimen number	Age days	Weight mg.	At 400 mm. Hg		At 760 mm. Hg		Ratio O ₂ at 400 mm. Hg O ₂ at 760 mm. Hg
			Frequency	Oxygen consumption	Frequency	Oxygen consumption	
			beats per minute	cu. mm. per gm. per minute	beats per minute	cu. mm. per gm. per minute	
*2-181 ♀	8-9	2.51	10,760	341	10,340	310	1.10
*2-182 ♀	8-9	2.72	11,060	337	10,460	306	1.10
*2-211 ♀	12-13	2.22	11,020	404	10,710	391	1.03
*2-221 ♀	6-7	2.39	10,770	401	10,360	406	0.99
2-231 ♀	7-8	2.69	10,210	337	9,580	312	1.08
2-251 ♀	9-10	1.93	11,050	248	9,520	236	1.05
2-252 ♀	9-10	2.79	10,160	330	9,410	330	1.00
2-281 ♂	4-5	1.64	10,400	363	9,140	356	1.02
Average		2.36	10,680	345	9,940	331	1.05
			At 200 mm. Hg		At 760 mm. Hg		Ratio O ₂ at 200 mm. Hg O ₂ at 760 mm. Hg
3-11 ♀	5-6	2.17	12,220	263	10,450	315	0.83
3-12 ♂	5-6	1.60	12,480	467	11,270	473	0.99
3-21 ♀	6-7	1.63	12,740	348	10,120	266	1.31
3-71 ♂	3-4	1.64	11,770	377	10,080	441	0.85
*3-72 ♀	3-4	1.90	12,050	345	10,200	409	0.84
*3-81 ♀	5-6	1.93	12,040	289	10,340	333	0.87
*3-91 ♀	6-7	2.25	10,910	310	9,670	320	0.97
*3-92 ♀	6-7	2.74	11,670	343	10,480	358	0.96
*3-101 ♀	7-8	1.84	11,210	308	10,270	294	1.05
Average		1.97	11,900	339	10,320	356	0.96

* Specimens thus marked were flown first at normal pressure; the others were flown first at reduced pressure.

Volumes corrected to NTP.

taneously, are presented in Table 9. Since the purpose of these measurements was to learn what effect alterations in density might have on the output of power by the flying insect, the runs were made in an oxygen atmosphere. As indicated above, the initial frequency of wingbeat appears to be independent of oxygen tension, but this is not true of the frequencies observed during continuous flight. In atmospheres with a low partial pressure of oxygen, the rate of wingbeat decreases rapidly after the first few seconds, and flight is maintained for a shorter period than under normal conditions. The rate of oxygen consumption also is depressed (Chadwick and Gilmour, 1940; Davis and Fraenkel, 1940). In the present experiments with *D. virilis* the tension of oxygen was higher than that of moist air at normal pressure even in the runs at a total pressure of only 200 mm. Hg, so that the results may be considered merely in reference to density change.

The flights were held to relatively short durations in order to minimize complications due to progressive fatigue, which reduces the rate at which oxygen is consumed. This factor was further equalized in the averages by reversing the order of pressures used in half the cases. The results thus obtained at 200 mm. Hg and 400 mm. Hg did not show any significant change in the average rate of oxygen consumption during flight at these pressures in comparison with the performance of the same individuals at 760 mm. Hg, although the data at 200 mm. Hg indicate a possibly significant depression for 4 of the 9 flies tested.

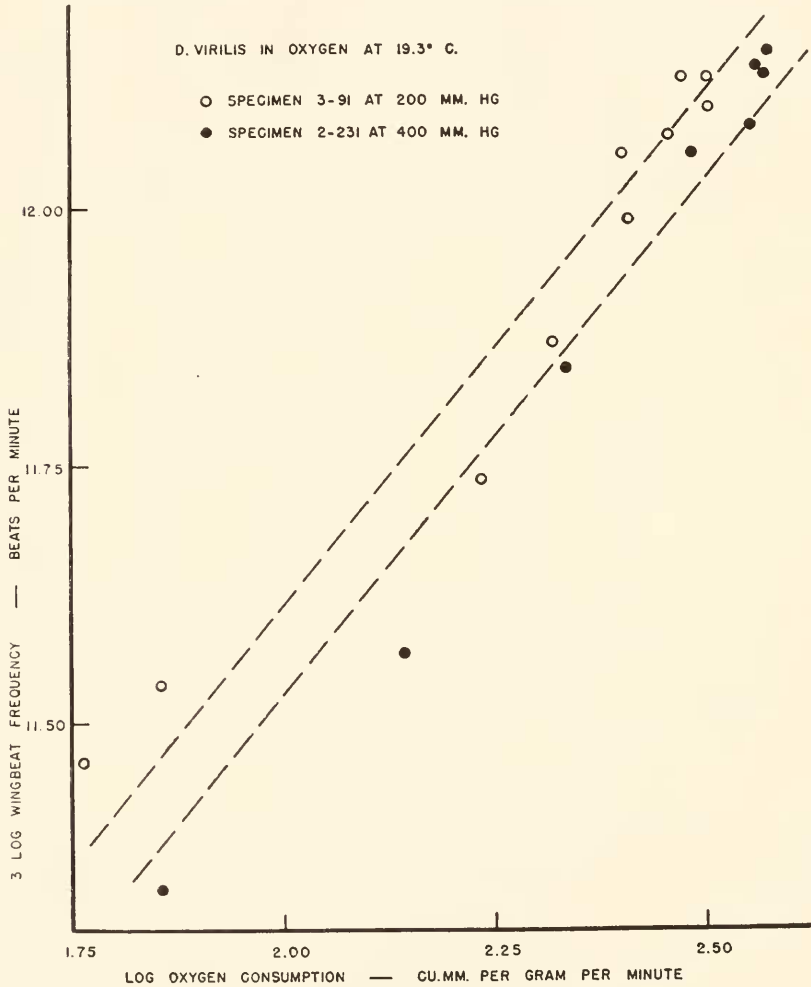


FIGURE 5. Correlation between wingbeat frequency and rate of oxygen consumption during flight in an oxygen atmosphere at reduced pressures. The broken lines have been drawn to conform to the equation, $3 \log F = \log k + \log O_2$, by using for $\log k$ the average value obtained for each specimen when the paired empirical values of wingbeat frequency and oxygen consumption were substituted into the above relationship. Each point represents the average values observed during 5 minutes of continuous flight.

The average oxygen uptake for 22 specimens, including some for which satisfactory runs were not obtained at reduced pressure, was 357 cu.mm. per gm. per minute at 19.3 degrees C. and 760 mm. Hg. These data agree closely with rates reported previously for flights of comparable duration with this species in air at 20 degrees C. (Chadwick, 1947). Thus it is apparent that the rate of oxygen consumption was not increased by supplying oxygen in excess of the tension normally present at one atmosphere.

In earlier studies a proportionality between the cube of wingbeat frequency and the rate at which oxygen is consumed (or CO₂ produced) was demonstrated for flights in air at normal pressure. Here, a few additional flights of about 40 minutes duration were made at 200 and 400 mm. Hg, and from them it was ascertained that the relationship

$$3 \log f = \log K + \log O_2 \quad (3)$$

applies at pressures other than normal. Typical results of such runs have been plotted in Figure 5.

DISCUSSION

The measurements reported above demonstrate that the principal factor concerned in the relationship between wingbeat frequency and atmospheric pressure is variation in gaseous density. A comparison of the values obtained in the helium

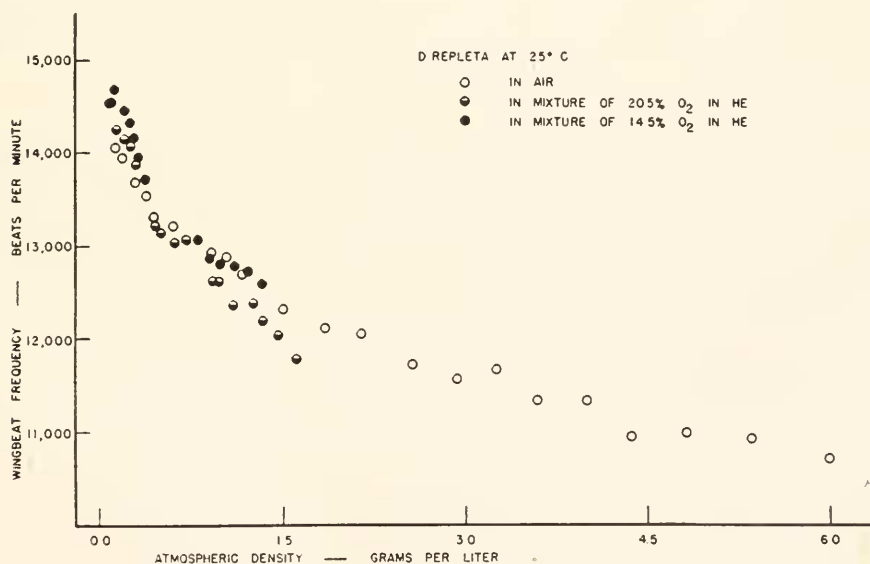


FIGURE 6. Wingbeat frequency of *D. repleta* as a function of atmospheric density in air and in two oxygen-helium mixtures.

mixtures with those obtained in air shows this clearly. In Figure 6 the three sets of data have been plotted together on coordinates where density, rather than total pressure, is the independent variable. Considering that the helium

detrimental effects upon the animals, and that there are in the curves irregularities due to various factors other than density, the agreement is convincing. Thus, at densities of about 1 gm. per liter, the average wingbeat frequencies in all the gas mixtures were identical within 2 per cent, although the determinations were made at less than one atmosphere in air, approximately $2\frac{1}{2}$ atmospheres in the 20.5 per cent oxygen in helium mixture, and at over 3 atmospheres in the 14.5 per cent oxygen in helium mixture. The failure of variations in oxygen tension to exert any effect, so long as the partial pressure remained above the limiting value of 15–20 mm. Hg, is also visible in these series, as it is in a comparison of results obtained in air and other oxygen-nitrogen mixtures. It is evident, therefore, that the correlation between wingbeat frequency and pressure depends, in fact, upon a relationship between wingbeat frequency and gas density.

A noteworthy feature of this relationship is the relatively small magnitude of the observed effects. Even when the density of air was increased five-fold by compression, the frequency of wingbeat decreased only 16 per cent. That this effect is indeed a minor one may be judged from a comparison of the effectiveness of change in gas density with that of change in environmental temperature. The decrement that 5 atmospheres of pressure produced in wingbeat frequency can, for example, be duplicated by lowering environmental temperature only 5 or 6 degrees C.

Such small effects of variation in gas density would not be anticipated on the basis of Equation (2):

$$f^3 \propto P/m. \quad (2)$$

The mass of air moved per beat is, obviously, equal to the product of stroke volume (V_s) and gas density (ρ). Hence

$$f^3 \propto P/V_s\rho. \quad (4)$$

If the power output (P) and the stroke volume (V_s) remain constant, then frequency (f) should vary inversely as the cube root of gas density; that is, as $\rho^{-0.33}$.

The large deviation of the actual relationship from this theoretical one is amply evident in Figure 4. Equations fitted by the method of least squares to the data obtained with *D. replcta* in air at 25 degrees C. are

for densities below 1.05 gm./L.,

$$\log f = 4.1271 - 0.0460 (\log \rho + 0.3529); \quad (5)$$

for densities above 1.05 gm./L.,

$$\log f = 4.0659 - 0.1028 (\log \rho - 0.4463). \quad (6)$$

With *D. virilis*, tested at two temperatures over the pressure range from 2 atmospheres to 200 mm. Hg, the relationships are:

at 19.3 degrees C.,

$$\log f = 4.0530 - 0.0885 (\log \rho + 0.0623); \quad (7)$$

at 25.9 degrees C.,

$$\log f = 4.1513 - 0.0726 (\log \rho + 0.0748); \quad (8)$$

and there is no evidence of any discontinuity.

In the latter respect, the true picture, we believe, is that presented by the studies with *D. virilis*. Here the samples of flies were more homogeneous, and all specimens were flown at all of the pressures included in the averages. Even so, differences were noted in the slopes of the curves given by different individuals. With *D. repleta*, where flies were selected at random from a mixed wild population, it happened by chance that more individuals whose performance yielded rate-density curves with small slopes were tested at the lower pressures, and more individuals giving greater slopes at higher pressures. Averaging these groups together has produced a composite curve with somewhat different slopes in the positive and negative pressure ranges.

With both species, the slopes for individual animals range from about -0.03 to -0.15 . These differences, which occur even in stocks reared under standard conditions, do not seem to be related to age or sex, and are not understood. It is unlikely that they depend on the factors involved in the correlation demonstrated by Reed et al. (1942), who showed that the rate of wingbeat is influenced by variations in the bodily dimensions which affect the ratio between muscle volume and the area of the wings. That this should be so is evident from Equation (4) above. Power output (P) will be proportional to the product of muscle cross-section and length; stroke volume (V_s), to the wing area. Hence, for a given air density, the wingbeat frequency will be less when the ratio P/V_s is small; i.e., when the wings are large relative to the power of the muscles which move them. But variation in the slope of the rate-density relationship cannot be ascribed to differences of this sort, for alterations in the ratio, P/V_s , should yield a family of parallel curves when the logarithm of wingbeat frequency is plotted against the logarithm of density.

Accepting these differences in slope as an unexplained phenomenon, we see nevertheless that whereas theory predicts the variation of f as $\rho^{-0.33}$, the actual measurements show f varying at a rate no greater than the -0.15 power of density. Since the theoretical relationship is based upon assumed constancy of stroke volume and power output, it is clear that one or both of these assumptions must break down when frequency changes in response to alterations in atmospheric density. Each of them must therefore be subjected to further examination.

In the absence of means for direct measurement of the power output of *Drosophila* at densities other than normal, we have turned to the rate of oxygen consumption as an index of this factor. The oxygen uptake gives a measure of the rate at which chemical energy is liberated by the active muscles and this figure, the power input (P_i), is related to P , the power output, through a factor, e , which represents the overall efficiency of the flight process:

$$P = eP_i, \quad (9)$$

Measurements reported above (Table 9) show that P_i is essentially independent of variation in density over the range from 200 mm. Hg to 760 mm. Hg, or suffers at most a slight decrease at the lower pressure. Apparently, the rate at which the muscles are able to liberate energy is limited largely by temperature and the physiological state of the insect in respect to fatigue, so that we may with reasonable safety extrapolate our findings at reduced pressures to cover the range of positive pressures in which measurements of oxygen consumption were not feasible with our apparatus, especially since we know that substitution of oxygen for air at normal

pressure is without effect on the rate of oxygen consumption during flight. If this reasoning is accepted, we may then conclude that the power output also should be independent of density provided that varying the latter does not cause changes in efficiency.

In attempting to decide this last question, we are again hampered by lack of data, for to settle the problem would require measurements of both power output and power input at several positive and negative pressures. One might perhaps anticipate some decrement in the efficiency of the wings at reduced pressures. Should this occur, it would help to account for the failure of wingbeat frequency to rise as rapidly as predicted by Equation (4) when density is decreased; but it seems very unlikely that a several-fold increase in efficiency occurs at a pressure of 5 atmospheres, as the logical extension of this argument to the range of positive pressures would demand. Since the relationship between wingbeat frequency and density for the individual insect is continuous, without change in exponent over the entire range tested, it follows that any compensatory alteration which would account for the divergence from a line of slope -0.33 must also be continuous. We are thus led to infer that changes in efficiency must be relatively unimportant when wingbeat frequency is altered as a function of density.

By elimination, then, we are persuaded to look upon changes in stroke volume as the most probable source of the compensation needed, and we must now inquire whether differences of the required magnitude are reasonably likely. Taking an average value of -0.10 for the slope of the empirical rate-density relationship, we may set

$$f^3 \propto \rho^{-0.30}, \quad (10)$$

and on substitution of this value into Equation (4) we obtain, with constant power output,

$$V_s \propto \rho^{-0.70}. \quad (11)$$

On this basis, if stroke volume at normal density is taken as 1, values of about 4.4 and 0.3 would be required at 100 mm. Hg and 3860 mm. Hg respectively.

Our judgment as to whether alterations of this size are within reasonable limits will be assisted by the following analysis. Consider that the volume, V_s , swept out by the wings in each cycle is approximately the segment of a cylinder. The radius of this cylinder is the wing length, L ; its height, h , is equivalent to the product of the average wing width, W , and the sine of the angle of attack, α :

$$h = W \sin \alpha. \quad (12)$$

The angle of attack is defined as the angle between the chord of the wing and the relative wind; thus the effective height of our hypothetical cylinder is the projection of the mean width of the wing on a plane perpendicular to its direction of motion relative to the oncoming column of air.

Now the volume of the segment swept out by the wings in a complete cycle, including both up and downstroke, will be related to the volume of a cylinder with the above dimensions as twice the stroke amplitude is to 360 degrees. Remembering that there are two wings, we may then summarize as follows:

$$V_s = 2\pi \times L^2 \times W \sin \alpha \times (2 \times \text{amplitude}/360^\circ), \quad (13)$$

or, since the wing dimensions are constant for a given specimen,

$$V_s \propto \text{amplitude} \times \sin a. \quad (14)$$

Thus we see that the principal variables involved in the stroke volume are the stroke amplitude and the angle of attack.

From head-on photographs of *D. repleta* in flight the amplitude at normal pressure in a plane transverse to the body axis has been measured as approximately 135 degrees with the wing tips making contact at the extreme of the upstroke. While this arc could theoretically be cut to 45 degrees to account for the required decrease in stroke volume at a pressure of 5 atmospheres, although the decrement in amplitude observed visually does not seem this large, the maximal extension possible (to a value somewhat above 180 degrees) would fall far short of supplying the 4-fold increase needed at 100 mm. Hg. For this reason it is apparent that the necessary changes in stroke volume must be effected in part through alteration in the angle of attack.

Since the stroke volume will vary with the sine of a , which for small values of the angle changes approximately as the angle itself, the hypothesis seems acceptable that the insect utilizes this mechanism in partial compensation for changes in air density. Lacking information to the contrary, we may conjecture in analogy with larger airfoils that the insect wing operates most efficiently under normal conditions with small values of a in the range from 0 degrees to 5 degrees, which are increased at densities lower than normal and reduced at higher densities. The same mechanism is familiar, of course, in the variable-pitch propellers of modern aircraft.

In summary, then, we may state that the wingbeat frequencies observed at densities other than normal are understandable only in terms of simultaneous variation in another element of the wing movement. A survey of the possibilities suggests that this must be the stroke volume, and that a part of the compensation derived from this source may be attributed to alterations in stroke amplitude, a reduction of which has been observed but not measured at higher densities. The larger share of the necessary variation in stroke volume comes apparently from small changes in the angle of attack. These have not been measured nor, since the direction of the relative wind must vary continuously as the wing sweeps through its complex path, does it seem likely that they will be. Power output and input, and the overall efficiency linking them, are probably essentially independent of variations in density.

The fact that the strain which results from alteration in the stress imposed by air resistance is distributed over several elements in the wing movement suggests analogies between the latter and other physiological functions in which homeostasis is observed. Wingbeat frequency changes less with alterations in density than if it alone were to compensate, while the deduced changes in stroke volume appear to be shared between alterations in amplitude and variations in the angle of attack. With regard to wingbeat frequency we know that it is governed largely by such physiological and environmental factors as substrate concentration (Williams, Barness and Sawyer, 1943), temperature, age and sex and we may imagine that these set a tempo of neuromuscular activity from which the organism has difficulty in departing even when confronted with major variations in other external influences. The way in which stroke amplitude and angle of attack may be regulated is unknown, but their dependence on density suggests the possibility of reflex con-

trol, mediated perhaps by campaniform receptors on the wings in response to variations in the amount of bending caused by air resistance.

SUMMARY

Wingbeat frequency of *Drosophila repleta* Wollaston was measured stroboscopically at 25 degrees C. as a function of atmospheric pressure, over the range from 100 mm. Hg to 3860 mm. Hg in air, in nitrogen-oxygen mixtures containing either more or less oxygen than air, and in two helium-oxygen mixtures. Similar measurements were made with *D. virilis* Sturtevant at 19.3 degrees C. and 25.9 degrees C. in air over the pressure range from 100 mm. Hg to 1520 mm. Hg; and at 760 mm. Hg, 25.9 degrees C., in a mixture of 6.1 per cent oxygen in nitrogen.

The flight response was inhibited when total pressure was less than 80 to 100 mm. Hg, or when the oxygen tension was less than 15–20 mm. Hg. Increasing the partial pressure of oxygen above the value for air did not increase the rate of wingbeat.

Within the limits of experimental error, the rate was found equal at equal densities, irrespective of the medium in which it was measured. Wingbeat frequency is therefore independent of total pressure as such, and varies inversely in a logarithmic relationship with the density. The exponents measured for this relationship varied with different individuals between -0.03 and -0.15 , approximately.

The helium-oxygen mixtures had a detrimental effect on the response of the insects, which was less evident at higher pressures and reversed when the specimens were returned to air.

Amputation of the halteres did not disturb the relationship between wingbeat frequency and density.

Clipping portions from the wingtips increased the frequency of wingbeat. When only one wing was clipped, the increase was less than when both were shortened by equal amounts.

Oxygen consumption of *D. virilis* was measured during flight in an oxygen atmosphere at 19.3 degrees C., at 760 mm. Hg, 400 mm. Hg and 200 mm. Hg, and was found to be relatively unaffected by variation in density.

Since wingbeat frequency varied less rapidly with changes in density than would be expected if both power output and stroke volume were to remain constant, it is reasoned that partial compensation is effected through adjustments in stroke volume. A decrease in stroke amplitude was observed at higher densities, but it appears unlikely that amplitude can increase enough at lower densities to account for the stroke volume required. Arguments are given to show that the remaining compensation needed may be furnished by alteration within reasonable limits of the angle of attack.

It is concluded that insect flight exhibits homeostatic characteristics, in that the strain which results from density change is distributed over several elements in the wing motion.

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